Recent studies of recombination in E. coli (17) have led to the discovery of a compatibility mechanism (15), a lysogenic system subject to genetic control (10), and a system of limited transduction by temperate phage (22) comparable to that of Salmonella (28). These three phenomena involve transfer of heritable factors by infection in contrast to bacterial mating which involves the entire genotype. The clarification, differentiation, and interrelationships of these mechanisms were emphasized in this investigation.

I The LYSOGENIC SYSTEM IN E. COLI K-12

The relationship of a temperate phage, λ, to a specific locus, Lp_1 (latent phage) has already been reported (10). In summary, the principal reaction types of bacterial strains are: sensitive (Lp^S), lysogenic (Lp^+), and the non-lysogenic resistant type, Immune-I (Lp^r). In crosses they behave as a system of multiple alleles, linked most closely with Gal_4. This linkage has been confirmed in a Gal^+ Lp^+ x Gal^- Lp^S cross in another laboratory (27). In addition, the two factors segregated out of heterozygous diploids in the parental coupling. This evidence points, therefore, to a genic determinant regulating the maintenance of λ provirus.

From a number of direct and indirect experiments it is known that all these types adsorb λ. A second locus, Lp_2, controls resistance or sensitivity to λ-2, a virulent λ mutant, and is situated in the Mal_1--S region of the chromosome. As Lp_2^r strains cannot adsorb λ, they are therefore not subject to any consequences whose initial reaction requires adsorption; Lp_2 does not interfere with the maintenance of λ previously established in Lp^+ strains. The genotype $Lp^sLp_2^r$ is consequently indistinguishable from $Lp^rLp_2^s$ types with respect to lytic effect of λ. Cross-reactions of λ with λ-2 antiserum have been observed.

New Data on Immune-1: The status of the various isolates of immune-1 strains has been reported, and the interpretation of their constitution with respect to prophage had been reserved pending evidence of a "cryptolysogenic" phage that normally fails to mature to give rise to lytic virus. The segregation pattern of $Gal^+Lp^+/Gal_4^-Lp^r$ diploids, also heterozygous for Mtl and Mal_1 (table 7) is identical with similar Lp^+/Lp^s results. The hypothesis that Lp^r types may carry a non-reproducing prophage is supported by experiments in which a low titer of λ was recovered by U-V induction of at least one (22). Lp^r types are also subject to transduction, and the results of these studies will be deferred to that section.

Incidental Variant Types: No new evidence bearing on the problem on the "semilysogenic" strain (10) can be presented. Tests to determine whether host-modified λ was carried (section III) were negative.

An intermediate host reaction, semiresistant to both λ and λ-2, comparable to the one in Shigella paradysenteriae (26) and the V_1^p allele of K-12 (11) has been clarified. Standard λ suspensions have a reduced efficiency of plating (eop) on this mutant such that the plaques produced are reduced in size and number, and also show a reduced efficiency of transduction. The mutants have been successfully lysogenized, but are still semiresistant to λ-2. The protocols for crosses which establish a mutation at a new Lp_3 locus not linked to Lp_2-Mal or Lp_1 - Gal, and conferring partial resistance to λ, are presented in table 13.

Mechanism of infection; Mutation and Selection vs. Induction: Breeding experiments and diploid segregations reveal only the chromosomal determinant of lysogenicity. The facility of the change Lp^s to Lp^+ encourages the possibility that λ directly induces (rather than selects) Lp^+ among the numerous survivors of exposure to phage. The following types of evidence would be useful in elucidating the primary infection process: (1) identification of a "prelysogenic" genotype in the absence of phage

would encourage the mutation hypothesis. It would be characterized as an apparent immune-1 that would be converted to a stable lysogenic after treatment with λ. (2) a careful study of the dynamics of infection, including the isolation of clonal pedigrees of single cells exposed to λ which engender lysogenics. A pure lysogenic pedigree would favor the induction hypothesis.

Attempts to identify the prelysogenic genotype in K-12, and hybrids of K-12 and other crossable lines have been unsuccessful. Preliminary experiments of the infection process (10) have disclosed lysogenic colonies contaminated with sensitive cells and free phage long after initial contact with λ. These mixed clones have since been confirmed in K-12 (18) and Salmonella (14,21,23B). The possibility that spontaneous alteration of the bacteria predisposing to a lysogenic decision plays some role in the recovery of lysogenics is thus not yet excluded. However, the simplest conception remains that the genetic elements of the phage are directly incorporated in, or attached to the bacterial chromosome as we have been able to find no indication of an extra-nuclear inheritance of lysogenicity.

The Effect of λ and F on Crossing Behavior: The presence of λ in one, both, or neither of the parents of a cross does not influence the yield of recombinants. As noted earlier (8) sensitives were not eliminated

as lethal phenotypes, but the progeny of lysogenic x sensitive included both parental types, and no others, in ratios dependent on the selected auxotroph markers. On the other hand, the compatibility factor (F) determines not only the yield but also the segregation pattern of many overtly unselected markers. Prototrophs are recovered only when at least one parent is F^+; F also seems to direct the elimination of certain chromosomal segments after the formation of the hybrid zygote (15,23). The important distinctions of F and λ are summarized in table 1. These are emphasized to mitigate any confusion that might arise from the suggestions that have been recorded elsewhere that λ may play a direct role in sexual recombination as well as to emphasize the distinction between the λ controlled transduction of restricted genetic factors and the F-controlled sexual recombination. The independent transmission of these factors was demonstrated by the recovery of (1) F^+Lp^S cells on the one hand, and F^-Lp^+ on the other, from mixtures of genetically labelled F^-Lp^S and F^+Lp^+, and similarly, (2) Lp^+F^- (but no Lp^SF^+ or Lp^+F^+) as survivors from F^-Lp^S exposed to λ-containing filtrates from F^+Lp^+ cultures.

II TRANSDUCTION

Cell-free filtrates derived from suitable Salmonella strains were capable of transferring unit genetic factors to a competent recipient (28). A wide range of independent markers has been equally subject to transduction. Additional analysis has shown that the temperate phage of the donor strain is the vector of the genetic material (16,25). Attempts to detect transduction in K-12 among the survivors in the turbid centers of λ plaques were negative (10); but by using high-titer lysates obtained by U-V induction (20), a successful transduction was achieved (22). Two striking contrasts with the Salmonella system were demonstrated: (1) the restriction to a single genetic character, galactose fermentation, and (2) a striking instability manifested by mosaic Gal^+/Gal^- colonies after transduction despite repeated single colony purification on EMB galactose agar. The incidence of persistent instability, rarely if ever encountered in Salmonella (14), varies with the recipient strain.

Confounding of Transduction with Recombination ?: The conditions required for transduction are generally precluded in crossing experiments. Moreover, the unstable mosaic Gal^+/Gal^- colony characteristic of transduction has not been so far recovered among recombinant progeny. A

more careful inquiry into the effect of λ and Gal segregation was necessary, however, in view of the transduction phenomenon, since it may provide an alternative interpretation of the Gal-Lp cosegregation ratios currently satisfied by a linkage explanation. Crosses of genetically related parents differing only in the presence or absence of λ were therefore studied. Table 2 demonstrates no significant deviation in the yield of Gal^+ recombinants where parents vary only for the Lp marker.

Is Transduction a Selection Artefact?: Interaction of genetic factors on reverse mutation of entirely independent loci have been reported before (15). An analysis of the Gal- segregation from the unstable transduction, the allelic transduction, reported below, as well as many other types of evidence (22) rule out the interpretation that the transduction is a selection artefact. The most convincing evidence, however, has been the development of specific Gal^- transductions in Gal^+ recipient strains by means of λ with extraordinary high frequency of transduction (22), when the λ donor was Gal^-.

Transduction and F-transfer: Just as lysogenization is independent of the conversion of F^- into F^+ strains, the transduction mediated by λ is unrelated to the F status of either the recipient or the donor cells.

Crosses of F^- x F^- by standard techniques are completely sterile. However, recombination of two nonallelic Gal^- mutants can be indirectly demonstrated by transduction. Lysates from $Lp^+Gal^+F^-$ were completely functional in introducing the Gal^+ factor to Gal^-F^- cells. Similarly, nonallelism of two Gal^-F^- strains can be established by the formation of Gal^+ in transduction experiments whereas the sexual sterility of the cross would block cell recombination in toto.

Crosses of a strain characterized by its enhanced fertility, Hfr, (15) displayed a linkage of the Hfr trait to Gal (12). These data were verified (table 3) for Gal^-_2. Despite this linkage, efforts to transport the Hfr and Gal^+ factors simultaneously into $Gal^-F^-Lp^s$ recipient cells via λ prepared from Hfr bacteria were unsuccessful. The conversion of F^- to F^+ by λ filtrates from F^+ strains was examined by crossing the Gal^+ transduction with F^- tester strains and was likewise unsuccessful. The competence of λ in transduction therefore continues to be confined to the Gal cluster.

The Concurrence of Transduction and Lysogenization: Observations on the E. coli system, as in Salmonella, are consistent with the hypothesis that the vector of transduction consists of temperate phage. As a rule,

the transductions isolated from Gal⁻Lp^S bacteria exposed to λ are consistently pure, stable lysogenics, despite the persistent instability of the Gal$^+$ trait; the ensuing Gal- segregants are also lysogenic. Lysogenization occurs very much more frequently than transduction, but the correlation of the two remained to be explored as evidence bearing on the hypothesis. In the first experiment (table 4 , part A) transductions were picked as Gal$^+$ papillae and streaked out on EMB galactose agar. A single Gal$^-$ (representing non-transinduced cells) and a single Gal$^+$ (the successful transduction) were each tested for lysogenicity on an appropriate Lp^S indicator. In experiment B, marked Gal$^+$Lp^S cells in the approximate proportions expected from transduction were introduced with the Gal$^-$ and the mixed culture on EMB galactose plates. With the assumption that both Lp^S strains would adsorb and be equally affected by λ, a disparity in lysogenizations of the two ensuing Gal$^+$ classes was looked for. Whereas all of the transduction Gal$^+$ were lysogenized, only up to 70% of the artifically inserted Gal$^+$ or of the original Gal$^-$ had been infected. Both parts of the experiment show a distinct correlation of lysogenization with transduction; the incidence of lysogenization is almost higher in these than in the control bacteria on the same plates.

Segregation of lysogenic sensitive has not so far been observed (up to 500 tests) from these simultaneously transduced and lysogenized recipients. This evidence argues that λ is the passive vector of genetic material from its source strain. This material is injected to the bacterium by the phage. In Salmonella the transduced genetic factors seem to undergo an immediate substitution for the homologues in the recipient bacterium, if they are successful at all. In E. coli K-12, however, an intermediate stage is perceived where one can detect simultaneously the presence of the original recipient and the new transduced genetic factors in the same cells by virtue of their subsequent segregation. The relationship between this replacement of genetic material and the conversion of virulent λ into its prophage stage ("reduction" 6) has not yet been completely worked out. As will be described below, however, these processes have been separated and are therefore not mutually dependent.

Lysogenization of Immune-1 in Transduction Experiments: When immune-1 strains such as W-1027 and W-1924 are exposed to λ, no evidence of their lysogenization is ordinarily perceived. However, under conditions where transductions can be selectively isolated about 5% of these altered bacteria

are also found to have been lysogenized. Repeated serial segregation of the resulting transductions showed that in some cases, lysogenicity failed to segregate. In others, lysogenicity and Gal segregate together, while in a single instance a lysogenic Gal$^-$ segregant was found which continued to segregate Lp^r colonies. Sometimes a very weak lysogenicity is observed ("one-plaque types" in cross-brush tests), which is completely lost after a few transfers. Some of these atypical cases are presented in table 5, and suggest the following alternative interpretations: (1) Lp^r cells are genetically lysogenic but carry a modified prophage. These cells are generally resistant to infection with λ. However, λ may be exceptionally introduced simultaneously with the Gal$^+$ fragment and there may displace the avirulent form of the prophage, or when Lp segregation is observed, both prophages persist together for the time being. (2) The Lp^r is a "null" allele. In transduction, Lp$^+$ and Gal$^+$ factors are introduced, but the lysogenic/immune segregation occurs when Gal segregates. This hypothesis can not account easily for the Gal$^-$Lp$^+$/$^-$ types except by devising a complicated scheme involving crossingover. (3) Immunes may or may not be genetically lysogenic. The production of Lp$^+$ signifies the occurrence of a double transduction at two loci, Gal and Lp. (a) ordinarily these linked factors would tend

to be lost as a block in the ensuing segregation, or (b) a linked transduction does not operate. By a two-step process, two effective particles have penetrated; one fragment carries Gal^+, the other Lp^+. Independent segregation is permitted and a mechanism requiring the breakage of a 2-factor linked fragment as in (2) is not called for.

In any event, special assumptions must be made on the avidity of the Lp^s locus for pro-λ to account for the failure of transductions to Lp^s to segregate Lp^+/Lp^s along with Gal^+/Gal^-. However, the Lp^r may only block the propagation of λ or its reduction to pro-λ.

Hypothesis (1) accounts for the occurrence of immunes which can be induced by U-V (22). The recovery of unstable Lp^+ transductions in non-transinduced Gal- would tend to support hypothesis 3. The most decisive elucidation of whether transduction displaces a mutant phage particle with a wild type λ or whether a normal Lp^+ allele is substituted for a mutant or null host Lp^r gene would be provided by experiments with genetically distinguishable λ preparations. Lp^r/Lp^s transductions were prominent with irradiated λ, tending to support hypothesis 2.

Irradiation effects: Quantitative assays of transducing potentiality of phage preparation are necessarily based on plaque counts. The survival

after various treatments of plaque-producing particles and transducing particles are not identical either in Salmonella (28) or K-12 (22). In fact, it is known from both studies that transducing power may be increased at some intermediate dosages. A comparison of the effects of U-V and X-radiation is given in table 6. A U-V dose reducing plaque assay from $1/2 \times 10^{10}$ to 16.9×10^{5} per ml yielded 170 transductions from an initial titer of 10^{3} / ml. A comparable X-ray dose was found to be between 150,000 and 200,000 r. No recognizable transductions were recovered at the latter exposure. Two viewpoints are indicated: (1) the lytic and transducing principles in λ are separable by their independent survival, and (2) avirulent λ particles are produced but they are damaged only to the extent of virulence for the host cell. Conclusive evidence favoring one or the other views of Lp^{r}, however, is not yet at hand. A decisive chemical and genetic separation of the transducing material from the virus particle has not yet been experimentally achieved, whether or not it is at all theoretically possible.

GENETIC DEFINITION OF THE GAL LOCI

Recombination: Attention was focused on galactose nonfermenting mutants because of the coincidence of the first recognized λ-sensitive

mutant in Gal^-_4 (W-518), and the subsequent observation of linked segregation of Lp and Gal_4 (10). Gal^- mutants have been isolated directly by inspection of surviving colonies after U-V treatment on EMB galactose agar and also as non-papillating variants of Lac^- mutabile recovered on EMB lactose agar plates. Interaction of Gal^- and Gal^+ on the phenotypic expression and reverse mutation of Lac_1 and Lac_7 alleles have been described (9). Recombination analysis provided the evidence for a cluster of four linked Gal loci (7). Gal_1 and Gal_4 show a very low order of crossovers. Preliminary data could only differentiate them on the basis of behavior in Het crosses; Lp and Gal_1 are both hemizygous, while Gal_4^+/Gal_4^- heterozygous diploids are readily obtained (table 7).

Transduction: Transduction tests reinforce standard allelism tests (table 8), and in fact have tentatively identified several new loci, now awaiting confirmation by recombination analysis. Whether the relative yield of Gal^+ transductions is proportional to the map distance between Lp and the Gal locus is in question. The results of large-scale allelism tests made available to date by new techniques to facilitate crossing are summarized in table 9.

The instability characteristic of the Gal^+ transduction results in the mosaic colony already noted and deserves further comment. Despite passage through a large number of serial single colonies, Gal- segregants are almost always thrown off. In transductions from Gal^+, i.e. $Gal^+ \rightarrow Gal^-$, these Gal^- segregants have been identified as alleles of the locus of the original recipient strain, both by crossing and further transduction tests. No other kinds of Gal^- have been recovered. On the other hand, if the donor is a non-allelic Gal^-, both donor and recipient Gal^- appear among the segregants from the Gal^+ transduction (22). For example, $Gal_2^- \rightarrow Gal_4^-$ gives galactose-fermenting intermediates, presumably of the constitution $Gal_2^- Gal_4^+ / Gal_2^+ Gal_4^-$. The segregants in all these tests are identified by (1) crossing experiments with Gal_2^- and Gal_4^- testers, (2) deriving λ and subjecting the testers to its action, and (3) applying λ from Gal^+, Gal_2^-, Gal_4^-, etc. The $Gal_2^- Gal_4^-$, a crossover type, has not been conclusively and consistently established. This double mutant would be identified as one which is subject to transduction by λ from Gal^+ and from any Gal^- other than Gal_2^- or Gal_4^-, and would yield no Gal^+ recombinants in crosses with Gal_2^- and Gal_4^- testers.

Diploid studies: The preceding evidence points to a chromosomal localization of the Lp lysogenicity determinant closely linked to a series of Gal loci. Evidence for the segregation of a prophage linked to the Gal_4 locus ruled out the possibility of a random distribution of cytoplasmic particles in cells carrying λ(10). These observations have since been extended to Gal_2 and Gal_4 hybrids (all heterozygous Lp^+/s), and also $Gal_4^+Lp^+/Gal_4^-Lp^r$ diploids (table 10). A study of such diploids segregating out distinguishable λ types is in preparation. Preliminary evidence also has been obtained elsewhere from crosses with lysogenic parents, one carrying a mutant λ (or one "doubly lysogenic") the other doubly sensitive, which yielded Gal/Lp progeny in parental couplings (1).

The mutational independence of Gal and Lp was also examined in the doubly homozygous diploid. Comparable experiments with the closely-Lac_1 and V_6 loci have already been reported. Lac^+ reversions were selected in $Lac^-V_6^r/Lac^-V_6^s$ diploids. The resulting doubly heterozygous diploids were of two types: $Lac^+V_6^r/Lac^-V_6^s$ and $Lac^-V_6^r/Lac^+V_6^s$, and with equal frequency (11).

A double homozygote $Gal_2^-Lp^s/Gal_2^-Lp^s$, also segregating a few other markers, (and unfortunately also Lp_2) was prepared by stepwise exposure of

the double heterozygote to U-V (14) and the isolation of suitable "reorganized" diploids. The resulting diploid, H-331 was infected with λ. Several $Gal_2^-Lp^+/Gal_2^-Lps$ isolations, A to G, were then allowed to papillate on EMS galactose agar. Independently occurring Gal^+ were selected, and the segregation pattern of Lp and Gal_2 of the resulting double heterozygotes was tested. The incidence of mutation to Gal^+ on the Lp^+ chromosome (coupling phase, or cis configuration) was compared with that on the Lp^s chromosome (repulsion phase, or trans-configuration). The analysis included a single Gal^+ and a single Gal^- segregant from a large number of diploids, (pair analysis) and the examination of many segregants from a single mass diploid culture (random analysis). From diploid B, 5 cis configurations and 6 trans configurations were scored. The conclusion from this evidence (table 11) is that the condition of the Lp locus, whether lysogenic or sensitive, has no significant bearing on which one of the 2 Gal^- alleles will mutate to Gal^+. (These preliminary data will be expanded, and also extended to a corresponding study of diploids first made heterozygous $Gal_2^-Lp^s/Gal_2^+Lp^s$, and then infected with λ.)

The above studies provide two kinds of Lp^{+}/Lp^{s}; Gal^{+}/Gal^{-} diploids: λ coupled on the one hand with Gal^{+} (cis) and on the other, with Gal_2^{-} (trans) If the activity of from "trans" bacteria is confined to non Gal_2^{-} recipient cells, a chromosomal but not nuclear limitation to λ specificity is indicated. All Gal^{-} including Gal_2^{-} is expected to respond to cis λ. A difference in λ from these diploids which are phenotypically identical, and genetically identical except for the arrangement of component parts established a "position effect." So far, only λ from the trans-type diploid has been prepared. Table shows that while Gal_4^{-} ($Gal_2^{+}Gal_4^{-}$) cells are subject to transduction, only rare Gal_2^{+} transductions were recovered. The development of an adequate diploid culture to satisfy the nutritional prerequisites for U-V induction in K-12 (3,5) and an intermediate growth period necessarily permits some selection for haploid segregants. The yield of λ obtained very probably includes a limited portion derived from $Gal_2^{-}Lp^{+}$ and $Gal_2^{+}Lp^{+}$ haploids. The latter crossover types may account for those transductions which were found. The data so far allow the tentative conclusion of a position effect hypothesis and strengthen the concept of an intimate relationship of λ and Gal at a specific action site on the chromosome. Transductions of the double homozygote M-331 and lysogenic

derivatives has apparently been obtained. The analysis is complicated by the fact that diploid-haploid instability can be confounded with transduction instability.

COMPARATIVE GENETICS OF Lp AND Gal IN OTHER LINES

Among the independently isolated crossable strains of E. coli (12) the wild type of three lines (28,47, and 51) were sensitive to λ carried by line 1. A fourth, line 31, threw off rough variants which were all λ sensitive. These strains occurred in nature as F^- but could be altered to F^+ by growth with K-12 or suitable derivatives. So far, at least one Gal^- mutant is subject to transduction. Preliminary intra-line-47 crosses established an Lp locus like that of K-12, and a Gal-Lp linkage. Very little mapping work has been completed among these strain, and the emphasis so far in these studies has been the genetic behavior of λ in outcrosses with K-12.

Sensitives of each line are readily lysogenized by K-12 λ but these lysogenics show a reduction of eop on K-12 sensitive indicators. This system is entirely analagous to host modification demonstrated for T2 (19) and λ produced by strain C (2). The terminology established for these systems will be used to describe the properties of our strains.

Thus lines 28,31, and 47 can be designated as λ* lysogenic or λ* sensitive. Line 1 sensitives are more resistant to λ* than to type λ. λ* can be introduced at low rates into λ sensitive hosts, but normal rather than λ* is recovered. Similarly, normal λ is converted to λ* after a single passage in λ* sensitive hosts. The four phenotypes are readily distinguishable in cross-brush tests as follows:

Example	Type	Reaction with: λ-sens. C bacteria	λ*-sens. B bacteria	λ	λ*
line 1 lysogenic	A	+	+	R	R
line 47 sensitive	B	-	-	S	S
line 1 sensitive	C	-	-	S	R
line 47 lysogenic	D	-	+	R	R

+/- = lysogenic or not; R/S = resistant or sensitive

Two major hypotheses can be tested by intercrossing these types:

I Lp controls all reactions: the types A-D are determined at a single locus.

II Lp controls lysogenicity/ sensitivity; another locus, Mp, controls resistance or sensitivity to λ*.

(a) Both λ and λ* are fixed at Lp in phenotypes A and D.

(b) λ is fixed at Lp in type A; λ* is fixed at Mp in type D.

The consequences of these hypotheses are shown in table 12. The critical crosses for I and II are A x B and C x D. The only decisive cross for II a vs. II b is A x D. II b would be favored by the recovery of sensitive recombinants as well as a novel genotype whose phenotypic effects are unpredictable. Since there is a possibility that Lp and Mp are closely linked a large sample of progeny many be required. One must bear in mind, in reviewing these intercross data that the prototrophs represent recombination of as yet unmapped nutritional factors. In addition, chromosome and other irregularities correlated with interstrain hybrids have not been analysed.

Effective transductions have been achieved in these strains. Gal- in lines 47 and 31 have been used as recipients, for λ produced by line 1, 28, 31, and 47. A reduction in the effectiveness of transduction to line 1 recipients is parallel with the reduced effectiveness of lysogenization. In general no important differences with the K-12 mechanism have been demonstrated. Hypothesis II b is doubtful.so far. The differentiation of the λ+ of different lines is still to be tested. A single intercross shows no genetic difference so far.

In preparing this report, it has been necessary to make numerous references to the unpublished work carried on in this laboratory by Professor J. Lederberg, Mr. M. L. Morse, and others, under other auspices. These are cited by number to the bibliography.

Table 1.

Characteristics of F (compatibility factor) and λ (virus)

	Criterion	F status	λ (effects)
(1)	Yield of recombinants	Decisive	None
(2)	Type of recombinants	Decisive	None
(3)	Transmission to recombinants	100%	Segregated according to linkage with selected nutritional markers; behaves as a genetic locus.
(4)	Transmission by infection	Rapid and fixed	Results in mixed clones (3).
(5)	Cell-free preparations	Not yet accomplished	Easily filtered.
(6)	Effect of antiserum	Slight if any	Blocks adsorption
(7)	Role in Gal^+ transduction	None	Decisive

Table 2

The Effect of λ on % Gal^- Progeny

M^-Gal^- parent	x	T-L-Th-Gal^+ parent lysogenic	sensitive	immune
F^+	lysogenic	8.0		7.1
	immune	6.3		6.3
	sensitive	6.7		10.1

Table 3

Linkage of Gal, Lp, and Hfr

W-1895 x W-2308

Part A:

Genotypes recovered[1]			Total
Gal	Lp	F	
+	+	+	14 *
−	s	−	29 *
+	s	+	5
−	+	−	0
+	s	−	4
−	+	+	0

Part B: 2 x 2 contingencies

	Gal^+	Gal^-	Total	F^+	F^-	Total
F^+	20*	0	20			
F^-	9	31*	40			
Lp^+	15*	0	15	13*	5	18
Lp^s	11	29*	40	6	33*	39
Lac^+	26*	5	31	22*	9	31
Lac^-	4	26*	30	7	27*	34
Vl^r	1*	9	10	1*	9	10
Vl^s	28	21*	49	23	20*	43
Xyl_2^+	9*	1	10	7*	2	9
Xyl_2^-	20	30*	50	16	7*	23

* Parental combination

1 Selected as Gal^+ and Gal^- prototrophs.

Table 4

Lysogenization in Transduced and Nontransduced Lp^s

Part A: Gal^+ and Gal^- from single papillae

Gal^+/Gal^- Pair type	Number
Lp^+/Lp^+	13
Lp^+/Lp^s	15
Lp^s/Lp^+	3
Lp^s/Lp^s	2
Lp^r/Lp^s	2
% Gal^+ sensitive	15.2
% Gal- sensitive	47.2

	Gal^- Lp^s	Gal^- Lp^+
Gal^+ Lp^s	2	3
Gal^+ Lp^+	17	13

Part B: Lysogenization of transduced and inserted Gal^+

Lp^s strains	Av. No. Gal^+ recovered: Control	Treated*	Types in mixture	No. tested	% lysogenic
Gal^+Lac^+	109	92	Gal^+Lac^+ (inserts)	46	68.5
Gal^-Lac^-	11**	432	Gal^-Lac^- (original)	40	72.5
Mixture***	106.5	419	Gal^+Lac^- (transductions)	103	100.

* 10^9 λ

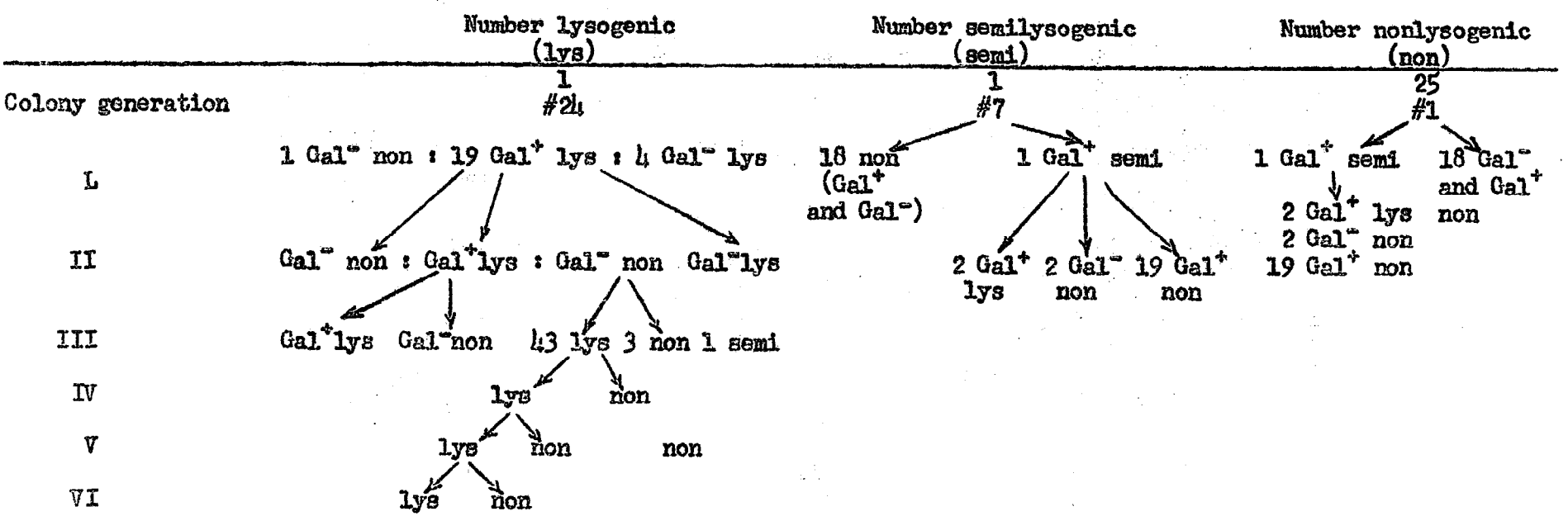
** Spontaneous reversions per 10^8 inoculum

*** 10^8 Gal-Lac- and 109 Gal^+Lac^+

Table 5

Transductions to Gal_4^- Immune-I: Segregation Patterns

Exp. 385: Strain 1924: 27 Gal^+



Exp. 431: Strain 2110: 38 Gal^+: 28 non, 1 semi (#23), and 9 lys

Segregation patterns of lys	all Gal^+ lys, all Gal^- non: 2 all Gal^+ lys, all Gal^- lys: 5 all Gal^+ lys, Gal^- lys and non 2 both Gal^+ and Gal^- non: #23

Table 6

Survival and Transduction with Irradiated λ

	No phage	Untreated phage	U-V[1]	X-ray[2] (x 10^3 r) 50	100	150	200
Av. plaques/ml x 10^5	0	127,000	16.9	41,667	3,975	377	100
% survival	-	100	0.013	32.8	3.13	0.297	0.008
Lp^S bacteria							
No. Gal^+ papillae	20	1,000	170	250	85	30	30
% " "	0.5	100	34	25	17	6	6
Lp^r bacteria							
No. Gal^+ papillae	39	60	-	135	115	31	20
% " "	65	100		225	191.7	5.2	3.3

1 20 minutes, sterilamp

2 10^3 r/min. at 250 K.V., courtesy A. Novick, Radiobiology Inst., U. of Chicago.

Table 7

Segregation of Gal, Lp,... diploids

A. H-324 Segregation of Lp_2, B_1, not tabulated.							B. H-325 Segregation of V_6, Mtl, Lp_2, B_1 not tabulated.	
Gal_2^-	Gal_2^+	Lp	Mal	Xyl	M	T,L	Gal_1^-	Gal_1^+
1	47	+	+	+	-	-	1	49
0	1	+	+	-	-	-	0	0
0	1	+	+	+	-	+	2	0
0	0	+	+	+	+	-	0	1
1	0	+	+	+	+	+	0	0
2	0	+	+	-	+	+	0	0
25	0	s	-	-	+	+	13	0
9	1	s	+	+	-	-	13	1
3	0	s	-	-	-	-	0	0
6	0	s	+	+	-	+	7	0
1	0	s	-	+	+	+	0	0
2	0	s	+	+	+	+	3	0
0	0	s	-	-	-	+	12	0
50	50	Total tested					51	51

Table 8

Allelic Specificity of the Gal - λ Transduction at the Gal 1, Gal 2, and Gal 4 loci.

λ- donor bacteria			Recipient cells		
Gal 1	Gal 2	Gal 4	1-2+4+	1+2-4+	1+2+4-
+	+	+	+	+	+
-	+	+	-	+	+
+	-	+	+	-	+
+	+	-	+	+	-
diploids:					
+ / +	- / +	+ Lp$^+$ / + Lp^s (trans)	No data	± (21)*	+ (300)*
+ / +	+ / -	+ Lp$^+$ / + Lp^s (cis)		No data	

* Gal + papillae per 10^9 λ

Table 9

Summary of Current Allelism Tests

Exp. No.	Gal^- type	F^- parent	F^+ parent	Total** progeny	No. Gal^+	Maxim. % Gal+
535*	1 x 4	W-750 Lp^+	W-2234 Lp^S	5000	17	0.3
563*				2000	15	0.75
534*	2 x 4	W-1210 Lp^+	W-2234 Lp^S	6000	25	0.4
563*				1600	11	0.68
580*				2400	8	0.3
535	4 x 3	W-518 Lp^S	W-2315 Lp^+	807	6	0.74
582	4 x ?	W-518 Lp^S	W-2315 Lp^S	5000	0	0
				6700	5	0.06
583	1 x ?	W-2291 Lp^S	W-583 Lp^+	7603	2	0.026

* All Gal^+ recombinants in these experiments are Lp^S.

**Estimated total.

Table 10

Behavior of Gal and Lp in Lac +/- Diploids

Type of cross		Parents F	(T L Th)	M	Lac_1	Lac_4	Gal_1	Gal_4	Lp	Diploid progeny Gal	Lp
1. Het diploids	(a)(Het)	+	-	+	+	+	+	+	+	+/-	+/∘ or -/∘ 1/ 5/
		+	+	-	-	+	+	-	s		
	(b)(Het)	+	-	+	+	+	+	+	+	+/∘ or -/∘	not segregating
		+	+	-	-	+	-	+	+		
2. $Lac1^-$ x Lac_4^-	(a)	-	-	+	+	-	+	+	+	Mostly +/∘	Mostly +/∘ 2/
		+	+	-	-	+	+	-	s		
	(b)	+	-	+	+	-	+	+	+	Mostly -/∘	Mostly s/∘ 2/
		-	+	-	-	+	+	-	s		
3. Haploid x auxotrophic diploid	(a)	- 4/	-/∘	+/-	+/-	-/+	+	+/∘	+/∘	$Gal^+ Lp^+$ / $Gal^- Lp^s$ (linked) 3/	
		+	+	-	-	+	+	-	s		
	(b)	same, except M- parent is Lp^r								$Gal^+ Lp^+$ / $Gal^- Lp^r$ (linked)	

1/ In Het crosses, Lp does not segregate. Gal 1 and Gal 4, two closely linked loci also differ: Gal 4 segregates, but Gal 1 does not.

2/ Diploids resulting from delayed disjunction revealed by heterozygotes of two Lac pseudoalleles show no segregation of Gal or Lp. Reversal of F status reverses the polarity of the Gal, Lp segregation.

3/ The only successful demonstration of heterozygosity of Gal and Lp.

4/ Aeration phenocopy.

5/ +/∘ indicates purity for +, whether hemizygous or homozygous.

Table 11

Segregation Patterns of Gal^+ Reversions in $Gal_2^-Lp^s/Gal_2^-Lp^+$ Diploids

Diploid number	Total segregants	Gal^+		Gal^-		Gal^+		Gal^-		Gal^+		Gal^-		Inferred type of diploid
		Lp^+	Lp^s	Lp^+	Lp^s	Lp_2^r	Lp_2^s	Lp_2^r	Lp_2^s	Mal^+	Mal^-	Mal^+	Mal^-	
A 1	161	76	6	3	76	45	0	39	0	1	53	17	36	cis
B 1	121	2	58	60	1	52	8	60	1	38	22	61	0	trans
B 2	73	0	40	41	0	32	7	31	0	33	7	33	0	trans
B 3	76	61	4	1	10	65	0	57	5	65	0	44	18	cis
C 1	48	1	23	24	0	23	1	24	0	9	15	24	0	trans
E 1	60	30	0	3	27	26	4	24	6	30	0	16	14	cis
E 2	24	0	12	12	0	12	0	12	0	6	6	12	0	trans
E 3	23	12	0	0	11	12	0	11	0	12	0	3	8	cis
F 1	66	32	1	2	31	31	2	30	3	32	1	21	12	cis
F 2	40	20	0	1	19	20	0	20	0	20	0	7	13	cis
F 3	23	12	0	0	11	12	0	10	1	12	0	3	8	cis
F 4	18	11	0	1	6	10	1	0	7	11	0	7	0	cis

Table 12

Genetic Determination of Host Modification: line 1 lines 28, 31, 47

		Genotypes Under				
		Hypothesis I Lp locus with alleles	Hypothesis IIa fixed at Lp, modified by Mp		Hypothesis IIb fixed at Lp in line 1, at Mp in other lines	
Phenotypes	Symbol	Lp	Lp	Mp	Lp	Mp
lysogenic	A	+	+	r	+	r
sensitive*	B	s*	s	s	s	s
sensitive	C	s	s	r	s	r
lysogenic*	D	++	+	s	s	+
A X B		None	C, D		C, D	
B X C		None	None		None	
C X D		None	A, B		A, B	
A X D		None	None		B and Lp^+Mp^+	

EXPTL. RESULTS:	Lines crossed	Type	A	B	C	D	Gal char.
Expt. No. 419	1 x 28	A Gal⁻ x B	0	46	1	0	+
			18	0	0	0	-
		C Gal⁻ x D	0	0	0	34	+
			2	8	18	3	-
418	1 x 31	A Gal⁻ x B	3	43	26	1	No record
420		A Gal⁻ x B	4	22	28	12	Gal^+ only
423		A Gal⁻ x B	8	2	1	37	+
			0	1	0	0	-
423		C x D Gal⁻	28	1	3	0	(and 28 Lp_2^r) B or C
444		C Gal⁻ x D	2	2	19	0	mostly Gal⁻
502		B Gal⁻ x C	0	15	13	0	+
			0	13	68	0	-
443	31 x 31	B x A	0	26	0	1	
468	1 x 47	A x B Gal⁻	51	0	0	6	+
			0	2	2	3	-
527		A Gal⁻ x B	4	7	1	9	+
			41	0	0	2	-
528		B x C Gal⁻	0	13	17	0	+
			0	8	24	0	-
529		C Gal⁻ x D	3	2	2	21	+
			2	2	28	0	-
523		A Gal⁻ x D	8	0	0	52	+
			37	0	0	19	-

F⁻ parent underlined.

Table 13

Genetic Control of the Semiresistant Phenotypes:

Nonlysogenic (W-2147) and Lysogenic (W-2172)

Part I

Hypothesis I: A new allele at Lp_2: Phenotype symbol	Lp_1	Lp_2	Example	Hypothesis II: A 3rd locus, Lp_3, is involved: Lp_1	Lp_2	Lp_3
A	+	s	Type lysogenic	+	s	s
B	+	r	Immune-2 lysogenic	+	r	s
C	+	p	W-2172 mutant	+	s	p
D	s	s	Type sensitive	s	s	s
E	s	r	Immune-2	s	r	s
F	s	p	W-2147 mutant	s	s	p

B x F Yields: B, F, E, C progeny Yields B, F, E, C, A, D
C x E

Results:	No. of Progeny B x F						No. of Progeny C x E					
	A	B	C	D	E	F	A	B	C	D	E	F
Mal^+	55	1	1	1	0	1	22	2	1	26	0	1
Mal^-	0	58	0	0	1	0	0	0	0	0	59	0

Part II Linkage of Lp_3 to Lp_1--Gal and Lp_2--Mal ?

Parents	No. of Progeny			
	$Mal^+ Lp_1^s$	$Mal^+ Lp_1^+$	$Mal^- Lp_1^s$	$Mal^- Lp_1^+$
F Mal^+ x B Mal^-	4	56	1	58
C Mal^+ x E Mal^-	27	25	59	0
	$Mal^+ Lp_2^s$	$Mal^+ Lp_2^r$	$Mal^- Lp_2^s$	$Mal^- Lp_2^a$
F Mal^+ x B Mal^-	59	1	0	59
C Mal^+ x E Mal^-	51	2	0	59
	$Mal^+ Lp_3^s$	$Mal^+ Lp_3^p$	$Mal^- Lp_3^s$	$Mal^- Lp_3^p$
F Mal^+ x B Mal^-	57	3	59	0
C Mal^+ x E Mal^-	50	2	50	0
C Gal^+ x D Gal^-	$Gal^+ Lp_1^+$	$Gal^+ Lp_1^s$	$Gal^- Lp_1^+$	$Gal^- Lp^s$
	60	0	0	28
	$Gal^+ Lp_3^s$	$Gal^+ Lp_3^p$	$Gal^- Lp_3^s$	$Gal^- Lp_3^p$
	37	23	37	26

The above data are consistent with the hypothesis that an Lp_3 locus separable from Lp_1 and Lp_2 modifies the reaction to λ-1 and λ-2. This locus is not linked to Lp_1--Gal or Lp_2--Mal.

REFERENCES

1. Appleyard, R. K. 1954 Segregation of lambda lysogenicity during bacterial recombination in E. coli K12. Cold Spring Harbor Symp. Quant. Biol. 19. In Press.

2. Bertani, G. and J. J. Weigle. 1953 Host controlled variations in bacterial viruses. J. Bact. 65: 113-121.

3. Borek, E. 1952 Factors controlling aptitude and phage development in a lysogenic Escherichia coli K-12. Bichim. et Biophys. Acta 8: 211-215.

4. Cavalli-Sforza, L. L. and J. L. Jinks. 1953 Observations on the genetic and mating system of E. coli K-12. Abstr. 9th International Congress of Genetics, Bellagio.

5. Gots, J. S. and G. R. Hunt, Jr. 1953 Amino acid requirements for the maturation of bacteriophage in lysogenic Escherichia coli. J. Bact. 66: 353-361.

6. Jacob, F., A. Lwoff, A. Siminovitch and E. Wollman. 1953 Definitions de quelques termes relatifs a la lysogenie. Ann. Inst. Pasteur 84: 222-224.

7. Lederberg, E. M. 1950 Genetic control of mutability in the bacterium Escherichia coli. Ph.D. Thesis, University of Wisconsin.

8. Lederberg, E. M. 1951 Lysogenicity in E. coli K-12. Genetics 36: 560 (abstract).

9. Lederberg, E. M. 1952 Allelic relationships and reverse mutation in Escherichia coli. Genetics 37: 469-483.

10. Lederberg, E. M. and J. Lederberg. 1953 Genetic studies of lysogenicity in Escherichia coli. Genetics 38: 51-64.

11. Lederberg, J. 1951 Genetic studies with bacteria. In: Genetics in the 20th Century. MacMillan: New York. pp. 263-289.

12. Lederberg, J. 1952 Cell genetics and hereditary symbiosis. Physiol. Rev. 32: 402-430.

14 Lederberg, J. Unpublished.

15 Lederberg, J., L. L. Cavalli, and E. M. Lederberg, 1952. Sex compatibility in Escherichia coli. Genetics 37: 720-730.

16. Lederberg, J. and P. R. Edwards. 1953 Serotypic recombination in Salmonella. J. Immunol. 71: 232-240.

17. Lederberg, J., E. M. Lederberg, N. D. Zinder, and E. R. Lively. 1951 Recombination analysis of bacterial heredity. Cold Spring Harbor Symp. Quant. Biol. 16: 413-433.

18. Lieb, M. 1953 The establishment of lysogenicity in Escherichia coli. J. Bact. 65:642-651.

19. Luria, S. E. and M. L. Human. 1952 A non-hereditary, host-induced variation of bacterial viruses. J. Bact. 64: 557-569.

20. Lwoff, A., L. Siminovitch, and N. Kjeldgaard. 1950 Induction de production de phage dans une bacterie lysogene. Ann. Inst. Pasteur 79: 815-858.

21. Kaplan, A. S. and A. Lwoff. 1953 Factors affecting the lysogenization of Salmonella typhimurium. VI Int'l Congress of Microbiology II:203. Abstract No. 545.

22. Morse, M. L. Unpublished.

23A. Nelson, T. C. and J. Lederberg. Unpublished.

23B. Parry, W. R. and J. Edwards. 1953 The induction of lysogenesis in Salmonella typhimurium. J. Gen. Microbiol. 9: 342-349.

24. Skaar, P. D. Unpublished.

25. Stocker, B. A. D., N. D. Zinder, and J. Lederberg. 1953 Transduction of flagellar characters in Salmonella. J. Gen. Microbiol. (In press).

26. Wahl, R. and L. Blum-Emerique. 1952 Les bacte ies semi-resistantes au bacteriophage. Ann. Inst. Pacteur 82: 29-43.

27. Wollman, E. 1953 Sur le determinisme genetique de la lysogenie. Ann. Inst. Pasteur 84: 281-293.

28. Zinder, N. D. and J. Lederberg. 1952 Genetic exchange in Salmonella. J. Bact. 64: 679-699.